Occurrence of Extended-Spectrum Beta-Lactamases Among Blood Culture Isolates of Gram-Negative Bacteria

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ABSTRACT

The purpose of this study was to determine the frequency of extended-spectrum beta-lactamases (ESBLs) in gram-negative bacteria isolated from blood cultures, and the susceptibility of these isolates to non-beta-lactam antibiotics. A total of 130 isolates from patients of all age groups were collected over a period of 4 months. ESBL-production was detected by Jarlier’s double disc diffusion synergy test (DDST) and by a modified version of this test based on reduced inter-disc distance. ESBL-producing strains were examined for the susceptibility to amikacin, chloramphenicol, ciprofloxacin, gentamicin and netilmicin. Ten percent of gram-negative isolates showed ESBL production by the modified DDST, and no isolates could be detected by Jarlier’s test. These isolates showed high susceptibility to ciprofloxacin (69%) and chloramphenicol (54%). Klebsiella pneumoniae was found to be the predominant ESBL producer. It was observed that the modified DDST is simple, practical and not technically demanding. It would be carried out in a busy clinical laboratory for the detection of ESBLs in our hospitals. (J Infect Dis Antimicrob Agents 2004;21:53-8.)

INTRODUCTION

In recent years, bacterial resistance to beta-lactam antibiotics has risen dramatically. In gram-negative bacteria, beta-lactamase production remains the most important contributing factor to beta-lactam resistance.1 Extended-spectrum beta-lactamases (ESBLs) constitute a growing class of beta-lactamases that have been found in the Enterobacteriaceae and Pseudomonas aeruginosa. The classification scheme of beta-lactamases by Bush, Jacoby and Medeiros places ESBLs in the functional group 2be capable of hydrolyzing oxyimino-cephalosporins (cefotaxime, ceftazidime and ceftriaxone) and/or the monobactams (aztreonam). They are commonly inhibited by beta-lactamase inhibitors.2

ESBLs are typically encoded on large 80-300 kb plasmids which can be exchanged between bacterial species resulting in cross-transmission, thereby spreading resistance among related and unrelated gram-negative bacteria. These plasmids may encode other antimicrobial resistance genes. Therefore, bacteria expressing an ESBL may express co-resistance to...
aminoglycosides, trimethoprim-sulfamethoxazole and tetracyclines and contribute to treatment failures, making it necessary to identify the prevalence of these strains in the hospitals.³

The present study was undertaken to determine the frequency of ESBLs in gram-negative bacteria isolated from blood cultures and to examine the susceptibility of the ESBL-producing isolates to non-beta-lactam antibiotics.

MATERIALS AND METHODS

This study included a total of 130 consecutive isolates of gram-negative bacteria recovered in pure culture from blood samples at the Department of Microbiology, Lady Hardinge Medical College and associated hospitals, Smt. Sucheta Kriplani Hospital (800 bedded) and Kalawati Saran Childrens’ Hospital (300 bedded). These are tertiary care hospitals situated in Delhi, India. The study period was for duration of 4 months from September to December 2001. The isolates were obtained from single blood culture specimen from patients of all age groups admitted to the intensive care units (ICUs), non-intensive care units (non-ICUs), nursery, neonatal unit (NNU), and from the out-patient department (OPD).

Bacterial isolates

0.5 ml-2 ml (for neonates and children) and 5 ml (for adults) of blood were collected and inoculated into glucose broth in blood culture bottles in a ratio of 1:10. After overnight incubation at 37°C, subcultures were done on sheep blood agar and MacConkey agar [Hi-Media Laboratories, Mumbai, India].

Antimicrobial susceptibility of the isolates was determined by the Kirby Bauer disc diffusion method as described by the National Committee for Clinical Laboratory Standards (NCCLS).⁴ The antimicrobial discs used included amikacin (30 g), ampicillin (10 g), amoxicillin+clavulanic acid (20 g+10 g), ceftazidime (30 g), cefotaxime (30 g), ceftiraxone (30 g), cefuroxime (30 g), chloramphenicol (30 g), ciprofloxacin (5 g), gentamicin (10 g) and netilmicin (30 g) [Hi-Media Laboratories, Mumbai, India]. Mueller-Hinton agar [Hi-Media Laboratories, Mumbai, India] plates were inoculated with the test inoculum adjusted to a turbidity of 0.5 McFarland standard. Plates were incubated overnight at 37°C. Quality control was performed by testing Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853. Susceptibility tests were interpreted by using the breakpoints as recommended by the NCCLS.⁴ The minimal inhibitory concentration (MIC) of cefotaxime [Hi-Media Laboratories, Mumbai, India] for all the isolates was determined by the agar dilution method.⁵

ESBL detection

The ESBL-producing ability of all isolates was determined by Jarlier’s double disc synergy test (DDST). In this test, the discs of expanded-spectrum cephalosporins (ESCs), namely cefotaxime and ceftazidime, and beta-lactamase inhibitor (amoxicillin+clavulanic acid) were placed at a distance of 30 mm (center to center). A modified version of this test was also carried out with the distance between the discs reduced to 15 mm.⁶⁷

Two Mueller-Hinton plates were inoculated with standard inoculum (turbidity corresponding to 0.5 McFarland) for each test organism. Cefotaxime and ceftazidime discs were placed on two sides of an amoxicillin+clavulanic acid disc in the center of the plate. The center-to-center distance between the discs was 30 mm on one plate and at a distance of 15 mm on the other. (Figure 1) The plates were examined after an overnight incubation at 37°C. If the isolate was an ESBL producer, the zone of inhibition around the cefotaxime/ceftazidime disc was extended towards the amoxicillin+clavulanic acid disc. E. coli ATCC 25922 was used as a negative control and an in-house ESBL-producing strain was used as the positive control.

RESULTS

One hundred and thirty isolates were obtained from blood cultures of patients (Table 1). There were 102 pediatric patients and 28 adult patients (73
The gram-negative bacterial isolates included *Klebsiella pneumoniae* (55, 42%), *E. coli* (19, 15%), *Acinetobacter* spp. (16, 12%), *Enterobacter aerogenes* (14, 11%), *Salmonella typhi* (12, 9%), *Salmonella paratyphi* (8, 6%), and *P. aeruginosa* (6, 5%) (Table 2). *S. paratyphi* and *S. typhi* were obtained from outpatients, while the remaining isolates were isolated from cultures of hospitalized patients.

All isolates belonging to the family Enterobacteriaceae except *Salmonella* spp. were resistant to ampicillin and amoxicillin+clavulanic acid (Table 2). Most isolates of *Salmonella* spp. were susceptible to all antibiotics tested. Four isolates of *S. typhi* were resistant to ampicillin and chloramphenicol and of these, 2 were also resistant to amoxicillin+clavulanic acid. Four isolates of *S. paratyphi A* were resistant to cefuroxime. The MICs of cefotaxime for the resistant isolates ranged from 64 to $>1,024$ g/ml whereas the MICs of control strains were $<0.5$ g/ml for *E. coli* and $8$ g/ml for *P. aeruginosa* (data not shown).

Jarlier’s DDST test did not detect ESBL production, while 13 isolates (10%) showed ESBL activity with the modified DDST test with reduced distance between discs. ESBL producers were
<table>
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<tr>
<th>Organism</th>
<th>Total numbers</th>
<th>55% (100%)</th>
<th>50% (91%)</th>
<th>55% (100%)</th>
<th>50% (91%)</th>
<th>53% (96%)</th>
<th>49% (89%)</th>
<th>54% (98%)</th>
<th>48% (87%)</th>
<th>37% (67%)</th>
<th>49% (89%)</th>
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<tbody>
<tr>
<td>K. pneumoniae</td>
<td>55</td>
<td>55 (100)</td>
<td>50 (91)</td>
<td>55 (100)</td>
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<td>48 (87)</td>
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<td>6 (32)</td>
<td>19 (100)</td>
<td>14 (74)</td>
<td>14 (74)</td>
<td>6 (43)</td>
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<td>10 (53)</td>
<td>15 (79)</td>
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<td>Acinetobacter spp.</td>
<td>16</td>
<td>8 (50)</td>
<td>5 (31)</td>
<td>5 (31)</td>
<td>5 (31)</td>
<td>7 (44)</td>
<td>11 (69)</td>
<td>10 (62)</td>
<td>8 (50)</td>
<td>4 (25)</td>
<td>5 (31)</td>
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<td>3 (50)</td>
<td>1 (17)</td>
<td>3 (50)</td>
<td>4 (67)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate percentage

*NT: not tested
detected in isolates from ICUs (1), NNU (7) and non-ICUs (4) (Table 3). The susceptibility pattern of ESBL producers to non-beta-lactam antibiotics was as follows: 31% (4/13), 31% (4/31), 38% (5/13), 69% (9/13) and 54% (7/13) isolates were susceptible to netilmicin, gentamicin, amikacin, ciprofloxacin and chloramphenicol, respectively (Table 4).

### DISCUSSION

Several studies have assessed the incidence of ESBLs in the Enterobacteriaceae. Most have focused primarily on *E. coli* and *Klebsiella* spp., of which the incidence of ESBLs is most common. In our study, ESBL production was determined in the Enterobacteriaceae and other gram-negative bacteria isolated from blood cultures.

The predominant gram-negative bacteria were *K. pneumoniae* followed by *E. coli*, *Acinetobacter* spp., *E. aerogenes*, *S. typhi*, *S. paratyphi* and *P. aeruginosa*. This is similar to a study from Southern India, which reported *Klebsiella* spp. to be the predominant gram-negative bacteria isolated from bacteremic neonates followed by *E. coli* and *Acinetobacter* spp. Another study from Northern India reported *K. pneumoniae* to be the most common isolate, but unlike the present study the next common isolate was found to be *Salmonella* spp. followed by *Pseudomonas* spp., *Acinetobacter* spp., *E. coli* and *Enterobacter* spp.

It is necessary to determine the prevalence of ESBL-producing isolates in a hospital to formulate the policy of empirical antibiotic therapy. This is important in the NNUs and the ICUs where a high percentage of infections are caused by resistant organisms. Although most isolates (69%) in the present study showed resistance to ESCs with high MIC values to cefotaxime
(64 - ≥1,024 g/ml), only 10 percent showed ESBL activity (14% of isolates resist to ESCs). Resistance to ESCs is explained by several mechanisms e.g. the expression of an Amp C-like beta-lactamase or expression of TEM-1 or SHV-1 beta-lactamase or associated alterations in the outer membrane permeability or the presence of a unique inhibitor-resistant beta-lactamase.14

ESBL-mediated resistance goes undetected unless specific tests for detection are used.3,8,9 Among the 13 isolates producing ESBL in the present study, 3 (22%) were found to be susceptible to cefotaxime and ceftazidime on disc diffusion testing. Various laboratory tests are available for detection of ESBL activity. We used the DDST test and found the zone of enhancement only when the distance between the amoxicillin+clavulanic acid disc and ESC disc was reduced to 15-mm and not at 30-mm which was recommended by Jarlier.7 This is similar to a report by Khurana et al who used various modifications of the DDST test and found that 15-mm distance gave the best results.15

In the present study, ESBLs were detected in a total of 13 (10%) isolates, which included K. pneumoniae, E. coli and E. aerogenes. ESBL production could not be demonstrated in the other gram-negative bacteria, possibly due to the limited number of isolates. A study from the USA reported 9.3 percent of clinical isolates belonging to the Enterobacteriaceae to be ESBL producers.16 A Belgian study reported a much higher prevalence of ESBLs (38.4%) among blood culture isolates of E. coli and K. pneumoniae.9 A tertiary care hospital in India reported a 68-percent prevalence of ESBL producers among gram-negative bacterial isolates, though the number of organisms that were resistant to ESCs but did not show ESBL production have not been mentioned by them.17 In the present study, only 14 percent of the isolates resistant to ESCs showed ESBL production and a study from Southern India reported 7 percent of resistant isolates were ESBL producing.11

ESBL-producing K. pneumoniae strains were reported to be more prevalent than other ESBL-producing gram-negative bacteria.15,17,18 In the present study, ESBL activity was demonstrated among 18 percent of K. pneumoniae followed by 10.5 percent of E. coli and 7 percent of E. aerogenes. Similar rates of ESBL-producing K. pneumoniae isolation (18%) was reported from West Indies.19 A Russian study reported a prevalence of 61 percent ESBL-producing K. pneumoniae.20 Villegas et al reported 33 percent of K. pneumoniae and 12 percent of E. coli to be ESBL producers.21 Sixty-two percent of ESBL producers in our study were isolated from NNU and ICUs. In a study from France, most of ESBL-producing gram-negative isolates (31%) were recovered from the ICU.6

In the present study, non-beta-lactam antibiotics with the greatest activity against the ESBL-producing isolates were ciprofloxacin (69%), followed by chloramphenicol (54%). This is similar to a study from Saudi Arabia, which reported ciprofloxacin (72%) had the greatest activity against ESBL-producers followed by amikacin (70%) and gentamicin (56%).22 A report from India showed the highest susceptibility of ESBL-producers to amikacin (87%).15

Currently, the NCCLS recommends an initial screening by testing for bacterial growth in a broth medium containing 1 g/ml of one of ESCs and further confirmed by determining the MICs of either cefotaxime or ceftazidime with and without clavulanic acid (4 g/ml).2 These procedures are recommended only for E. coli, K. pneumoniae and K. oxytoca. The recommendations of NCCLS may not be feasible to perform in many routine clinical laboratories. The modified DDST is a simple, practical and low-cost test, which can be carried out in busy clinical laboratories.

In conclusion, as the use of expanded-spectrum cephalosporins is becoming common in clinical practice and reported treatment failures are increasing, detection of ESBLs should be introduced in laboratories to
ESBL producers among blood culture isolates: Kapoor L, Deb M.

References


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spectrum beta-lactamase producing organisms at the University Hospital of West Indies. West Indian Med J 2004;53:104-8.

